Aflatoxins in Animal and Human Health

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I. Introduction

Although aflatoxins have been the cause of numerous deaths in livestock and suspected in some deaths of humans, the impact of aflatoxin consumption by a wide variety of animal species, including humans, extends much further than such a focal point. The economic significance of this group of toxins occurring in feeds and foods is through lowered productivity including meat and eggs, reduced weight gain, reduced feed efficiency, increased incidence of disease because of immunosuppression, and subtle damage to vital organs. Several human diseases have been recognized as being caused by aflatoxins, especially acute aflatoxicosis associated with highly contaminated foodstuffs (Shank 1977; Krishnamachari et al. 1975a, 1975b; Ngindu et al. 1982).

The modes of action and multiplicity of effects in humans and other animals are sufficiently complex, and in some cases obscure, to adequately provide prophylactic or therapeutic measures for aflatoxicosis. The intent of this overview is to provide the reader with an understanding of these effects

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based on experimental investigations or naturally occurring disease in humans and animals caused, at least in part, by aflatoxins.

A. Brief Historical Overview

The toxicity of a group of closely related compounds, called aflatoxin in the popular literature and often in the scientific press, was recognized in 1960 following one of the most intensive and productive investigations of unknown toxic factors of natural origin ever pursued. Peanut meal imported to Great Britain from Brazil was quickly targeted as the cause of the deaths of large numbers of turkey poults and ducklings on British farms. Mold hyphae were observed during examination for the presence of poisonous plants, although attempts at culture were negative (Goldblatt 1969). The disease syndrome was subsequently recognized in other domestic animals and in places other than England. The causative mold, *Aspergillus flavus*, that reproduced the animal disease signs and symptoms was actually isolated from meal associated with a hepatic problem occurring in ducklings in Uganda (Sargeant et al. 1961). The early history of the turkey outbreak in Great Britain was described by Blount (1960, 1961) and toxicity occurring in several farm species summarized by Allcroft (1965).

The identification of aflatoxins first utilized a bioassay with the duckling, a highly susceptible species, in which the proliferation of bile duct epithelial cells was clearly visible within a few days of the ingestion of toxic meal. It was soon discovered that the extraction of toxic meal yielded a highly fluorescent material that could be estimated visually and corresponded with the toxicity of the samples as determined biologically (Goldblatt 1969). The isolation and characterization of the four closely related toxins, aflatoxin B_1, B_2, G_1, and G_2, were first reported by Hartley et al. (1963), but their structures were not defined until 1965 by Buche and coworkers (Asao et al. 1965).

In early studies in 1961 with laboratory rats, liver tumors were noted (Lancaster et al. 1961) and came to the attention of the World Health Organization (FAO/UNICEF) who were considering peanut meal as a protein supplement in foods for undernourished children. Max Milner of UNICEF convened a meeting of interested scientists in New York City in October 1962 to help assess the hazards of aflatoxin to human health (Stoloff 1977; Wilson 1978). Also in the United States, an outbreak of trout hepatoma was recognized in the Spring of 1960 that dramatized and focused interest on the practical problems (Kraybill and Shimkin 1964), thus helping to set the stage for the extensive protracted search for the true role of aflatoxins in human liver disease (Goldblatt 1969).

W.H. Butler, in the proceedings of a symposium held at MIT in 1964, prophetically stated that "the carcinogenic properties of aflatoxin are now well documented, that large-scale epidemiological studies will probably be required to demonstrate the role which these natural toxins play in the etiology of liver cancer," and, finally, "that the presence of traces of compounds such as mycotoxins in food warrants considerable attention as a health hazard to man." However, Oettle (1965) is credited with being the first investigator to draw serious attention to the fact that aflatoxin ingestion might cause human liver cancer.

II. Human Aflatoxicosis

Supporting the concern for the possible carcinogenicity of aflatoxin was the fact that the rat and rainbow trout were found to be highly susceptible to aflatoxin tumorigenesis. However, the excretion pattern of the rhesus monkey was found to be different from that of the rat, and results in primates were scarce and only proved they were much less susceptible to aflatoxin tumorigenesis than rats. Adamson et al. (1973) initiated a mixed sex and species study involving 40 rhesus and cymologus monkeys. By 44 mon of age, one female who had received up to 800 μg/kg three times every 2 wk became progressively jaundiced and inactive, was killed, and primary liver carcinoma was observed. Campbell and Stoloff (1974) collated the primate studies, and their discussion emphasized the variability in results of these studies. (See also the section on carcinogenicity in animals.)

In 1966–67, prospective human epidemiology studies were initiated in Africa when Alpert and coworkers (1969) surveyed the incidence of primary hepatoma in Uganda and determined aflatoxin levels in food samples stored for consumption between harvests (Alpert et al. 1969). The frequency of aflatoxin contamination of market food samples was positively associated with liver cancer incidence in localized population groups.

Coincident with the considerations of the possible role of aflatoxins in hepatocellular carcinoma was an effort to tie it to other distressing human illnesses of unknown etiology, i.e., kwashiorkor and Reye's syndrome. There is no generally agreed on definition of kwashiorkor, but edema associated with hypoalbuminemia is universally accepted as the essential minimum criterion for diagnoses (Hendrickse 1984). Kwashiorkor was first thought to be caused by protein deficiency in the presence of relative carbohydrate excess. When this hypothesis became untenable, it was noted that animals receiving aflatoxin experienced several effects consistently found in kwashiorkor, i.e., hypoalbuminemia, fatty liver and immunosuppression, and that "the world distribution and seasonal fluctuations of mycotoxins contamination of food are remarkably similar to the geographical distribution and seasonal presence of kwashiorkor which tends to peak in the wet season in many areas" (Hendrickse et al. 1983).

For example, aflatoxin AFB_1 was detected from autopsy liver specimens in all 36 children who had kwashiorkor (Hendrickse 1985). However, Hen-

drickse (1985) concluded only "that our findings in these studies establish an association between aflatoxins and kwashiorkor. The nature of this association is obscure and is being investigated." Frustration from the failure of medicine to prevent and reliably treat kwashiorkor made the association with dietary aflatoxin easy to take to the next step of a cause-and-effect relationship.

Reye's syndrome, an encephalopathy with fatty degeneration of the liver, was described by Reye et al. (1963). Following scattered reports of aflatoxin in tissues of victims of Reye's syndrome, Ryan et al. (1979) using formalin fixed tissue found an extremely strong association between children dying with Reye's syndrome and aflatoxin isolation from liver. However, Rogan et al. (1985), following an investigation by the Centers for Disease Control, concluded that "we do not believe that most or many cases of Reye's syndrome in the U.S. are caused by aflatoxin exposure; right now we think it prudent to view reports of aflatoxin in the tissues as indicators of exposure of uncertain significance for etiology." In another search for associations, the Office of Rural Health in Georgia (Caster et al. 1986) reported that the consumption of large amounts of corn, rice, peanuts, and milk (food considered to be potentially high in aflatoxins) was significantly related to the mental retardation of children in one county having high levels of aflatoxins in the food supply. No such relationship was found in another county having trivial levels of aflatoxins (<20 ppb) in its diet.

Acute toxic liver injury in humans has been ascribed to aflatoxin. The most extensive and widely publicized case was an outbreak in Western India in October 1974, where unseasonal rains resulted in extensive mold damage to corn crops with extremely high concentrations of aflatoxin found to be 6.3–15.6 ppm in corn from affected households. The illnesses were characterized by jaundice, rapidly developing ascites, portal hypertension, and a high mortality rate. Dietary calculations and the analysis of contaminated samples showed that affected people could have consumed between 2 and 6 mg of aflatoxin daily over a period of a month. Extensive bile duct proliferation, periportal fibrosis, and occasional multinucleated giant cell, typical of aflatoxin-induced disease in animals, were found in the histopathologic examination of liver specimens (Krishnamachari et al. 1975b).

There may be inhalation exposure to aflatoxin among industrial and agricultural workers. The presence of aflatoxin in dust from contaminated corn has been established (Burg and Shotwell 1984; Burg et al. 1981; Silas et al. 1987). Because of the sporadic distribution of aflatoxin in corn and other contaminated commodities, it was difficult to accurately quantitate the contamination. In industrial settings, two Czechoslovakian chemical engineers died working on a method for sterilizing peanut meal (Dvorackova et al. 1981) and two British biochemists developed adenocarcinomas of the colon following exposure to purified aflatoxins (Deger 1976). Van Nieuwenhuize et al. (1973) identified a number of cancer cases among a small group of workers in a peanut processing plant in The Netherlands and associated them with the finding of aflatoxins at 300–400 mg/kg in the peanut meal. Hayes et al. (1984) conducted a follow-up study of workers in this plant. The test subjects extracted oil from peanuts and linseed oil, whereas the controls worked in a nearby corn processing plant and, because of the handling equipment in use, probably had much less dust exposure. The observed mortality due to cancer was reported to be higher than expected for the aflatoxin-exposed workers throughout the entire study period. However, the tumors were at a variety of sites with no primary tumors of the liver, but there was a much greater than expected number of tumors of the respiratory system in the aflatoxin-exposed group, with seven such deaths. The extent to which the development of tumors at individual sites was associated with aflatoxin exposure could not be determined.

There are several widely cited epidemiological studies attempting to link hepatocellular carcinoma with high intakes of aflatoxin B_1 from the diet. In the early group of studies, aflatoxin intakes were sampled in populations of different geographical areas and the results correlated with PLC incidence in those areas. These studies were by Shank et al. (1972) in Thailand, Peers and Linsell (1973) in Kenya, Van Rensburg et al. (1974) in Mozambique, Peers et al. (1976) in Swaziland, and Van Rensburg et al. (1985) in Mozambique/Transkei. In each of these locations except Mozambique, multiple areas were studied and aflatoxin exposure assessed by the measurement of dietary intake. Intake was not verified by measuring metabolites, DNA, or protein adducts in the urine of subjects.

Problems preventing strict adherence to good protocol design in these studies are discussed by Wagstaff (1985, 1990). These include short-term migration of working-age males in Africa, nonavailability of subjects, and difficulties in carrying out reliable analyses. In Kenya (Peers and Linsell 1973), the male population over 15 yr of age was 29% less than the female population of that group and the only liver cancer cases recorded were those people who voluntarily presented themselves to a hospital or clinic. The three study groups were not comparable, e.g., there were differences in altitude of the respective regions, proximity to the hospital, economic and agricultural conditions and crops grown, and diets. In Thailand, snacks and foods in short supply in homes, such as meats, were not sampled. Difficulties in case finding were so great in the Thailand study that incidence data in rural study areas were actually taken from nearby urban areas. Hepatitis B virus (HBV) status was not determined in any of these studies, although the possible role of HBV infection was discussed by Van Rensburg et al. (1985). Thus, with the exception of this latter study from Mozambique, the thesis was that aflatoxin might be the primary causative factor in areas of high primary liver cancer (PLC) incidence and, in fact, all the stud-

ies did demonstrate a positive correlation between aflatoxin intake and PLC incidence. However, in the United States, Stoloff (1983) found a small age-adjusted excess PLC mortality ratio for the Southeast and he questioned whether this small excess could be attributed to aflatoxin.

The early retrospective epidemiology studies depended on the accuracy of dietary surveys and analyses to establish aflatoxin consumption in the various populations surveyed. In addition, they assumed no infectious complicating factors in the etiology of PLC. In the years since the round of epidemiology studies were initiated in 1970, important advances have been made both in (1) identifying alternative etiologies and (2) tracking aflatoxin consumption. Epidemiological studies can now be carried out on a more scientific basis, thus assuring more confidence in their results.

The initial finding leading to the development of alternative etiologies for PLC was that of Sherlock et al. (1970), who noted the high prevalence of hepatitis B surface antigen (HBsAg) in a clinical series of PLC patients. The evidence for the association of PLC and HBV, aflatoxin and other possible risk factors has been summarized by Wagstaff (1985) and Bassendine (1987). Of importance in evaluating the role of HBV are such factors as the ecologic association of PLC and HBV in diverse regions throughout the world and case control studies comparing the prevalence of HBV serologic markers in PLC cases and controls. Any serious epidemiological study of PLC must now include testing for the HBsAg antigen to allow a concurrent evaluation of HBV with other risk factors in the test populations.

In the early studies, aflatoxin ingestion had to be estimated by taking appropriate samples of the diet and then assaying them by a reliable method before the aflatoxin content changed. Understandably, there was much room for error in this procedure. Confirmation of aflatoxin ingestion has now been made possible by the elucidation of its metabolism and detoxification pathways, identification of adducts, and development of rapid, sensitive, and accurate methods for adduct identification summarized by Groopman et al. (1988). An early finding was that of Campbell et al. (1970), who reported that aflatoxin M_1 was detectable in the urine of people who consumed more than 30 mg AFB_1/d. Later, after it was recognized that reactive electrophilic epoxides of many xenobiotics can covalently react with various nucleophilic centers in macromolecules, like DNA, RNA, and protein, such a pathway was also recognized for aflatoxin. The first major AFB_1-DNA adduct was identified by Essigmann et al. (1977) in an *in vitro* system; however, its presence was soon confirmed *in vivo*. The half-life of this adduct is approximately 12 hr, which severely limits its usefulness in confirming aflatoxin ingestion. Autrup et al. (1983) reported the presence of a putative AFB_1-Guanine product in urine collected from individuals living in areas with high liver cancer risk and suspected exposure to aflatoxin.

Aflatoxin B_1 binds to proteins as well as to DNA. The reaction with one or more lysine residues in serum albumin accounts for more than half of the total binding to this protein (Sabbioni et al. 1987). This adduct is a completely modified aflatoxin structure retaining only the coumarin and cyclopentene rings of the parent compound. Protein adducts are significant in biological monitoring because they represent the integrated dose of aflatoxin received over many previous weeks. The average half-life of albumin in humans is 20 d; thus, an accumulated dose of aflatoxin will be present after dietary exposure has ceased (Groopman et al. 1988).

Monoclonal antibodies are successfully used as the basis for tests to determine aflatoxin in foods and biological samples. These fast, relatively inexpensive, and easily performed tests allow the practical monitoring of human exposure to aflatoxin in field epidemiological studies (Groopman and Donahue 1988 and Garner et al. 1985). Monoclonal antibody columns, coupled with HPLC, can quantify aflatoxin-DNA adducts in human urine samples obtained from environmentally exposed humans (Groopman et al. 1988). These tests require strict quality control standards, e.g., the use of an internal standard is necessary for meaningful results; otherwise, variability in the assay can be high. The usefulness of this sensitive test still depends on adequate sampling of the diet of all test groups and the constancy of this diet over time, prospectively and retrospectively, as required by the particular protocol. A monoclonal antibody-based method to measure M_1 in urine was used by Sun et al. (1986) and Zhu et al. (1987) to document and quantify human exposure. Zhu found good correlation with diet only in male subjects.

The International Agency for Research in Cancer (IARC) has made several reports on the carcinogenicity of aflatoxin starting in 1976. This first report stated that "studies of liver cancer incidence in relation to aflatoxin intake provide circumstantial evidence of a casual relationship." However, by 1987, another IARC working group concluded that there was "sufficient evidence that aflatoxin is a probable human carcinogen." A later group of epidemiological studies—Sun and Chu (1984), Yeh et al. (1989), and Peers et al. (1987)—which reported an aflatoxin effect independent of HBsAg prevalence, were available to the latter working group and presumably influenced their conclusion (Campbell et al. 1990). However, the Sun and Chu report contained no aflatoxin intake data and the Yeh report compared only one "high" and one "low" village in the Guangxi region of China. In the third study, Peers and coworkers reinvestigated four regions in Swaziland studied earlier and were the first to utilize both food measurements of aflatoxin intake and HBV exposure of the same population. However, they had to estimate the consumption of peanuts, which were by far the most contaminated foodstuff, and the incidence of primary liver cancer was surprisingly low, being surpassed by cervical and esophageal cancer.

In another recent study, Autrup et al. (1987) reported no aflatoxin effect on PLC incidence rates when all ethnic, social, and cultural groups were combined but a positive association was noted among the Bantu people. Autrup et al. (1987) also measured HBsAg carrier rates. Yeh et al. (1989) reported on their study of HBV status, aflatoxin exposure, and PLC incidence conducted in southern Guangxi China between July 1982 and June 1987. In a cohort of 7,917 men aged 25–64 yr, a total of 149 deaths occurred, 76 of which (51%) were due to PLC. Prevalence of HBsAg positivity in PLC deaths was 91%, in contrast to 23% of all members of the cohort. However, the authors saw no HBsAg effect in the cross-sectional case analysis. There was an almost linear relationship between estimated aflatoxin B_1 exposure in the subpopulations and corresponding mortality rates of PLC. However, only the average regional aflatoxin exposure values were available and not the individual exposure values.

The latest epidemiology study to be reported that examined the association of PLC with dietary aflatoxin, among other factors, is fortunately far and away the most comprehensive, with 6,500 participants. This study by Campbell et al. (1990) was a comprehensive cross-sectional survey in the People's Republic of China on the possible risk factors for primary liver cancer. It included 48 survey sites, an aflatoxin exposure range of about 600-fold, a 39-fold range of PLC mortality rates, and a 28-fold range of HBsAg+ carrier prevalence. Although the accuracy of the urinary assay used for measuring aflatxoin metabolites has been questioned, this study did estimate exposures for a large number of nutritional, dietary, and lifestyle variables. It represents a much larger number of survey sites, a wider range of PLC mortality rates, and a wider range of aflatoxin exposure than in the previous surveys. In addition, aflatoxin consumption was estimated by measuring aflatoxin metabolites in urine.

In Campbell's study, in contrast to the reported results of some of the previous studies, there was no association between aflatoxin exposure and PLC mortality. There was also a lack of association of PLC mortality with corn and moldy peanuts, the two foods in the diet commonly contaminated with aflatoxin. However, PLC mortality was positively correlated to the prevalence of HBsAg and other factors. Notably, in the United States, Stoloff (1983) found a small age-adjusted excess PLC mortality ratio for the Southeast. However, there was serious question whether the excess PLC mortality could be attributed to aflatoxin.

A. Evidence from Analysis of Mutations

The interaction of chemicals or their metabolites with DNA is thought to be a major factor in chemical carcinogenesis. Tissue metabolism is important in the formation of nonreactive and reactive metabolites following exposure

to chemical toxicants. Some of the reactive metabolites formed in tissues can irreversibly bind to DNA. If the damaged DNA is not repaired and the cell subsequently divides, the damage can be fixed, resulting in a mutational event. Following mutation, a series of events is initiated, ultimately leading to an adverse biological effect such as cancer. This series of events is under investigation, with many suspect chemicals including aflatoxin.

In a recent series of papers in *Nature*, Hsu et al. (1991), Bressac et al. (1991), and Harris (1991) reported on the incidence of mutations in p53, a putative tumor suppressor gene in PLC from patients in the high-risk areas of southern Africa and China. The mutations in both areas were found to be clustered on codon 249 with transversions of G to C or to T. These base changes are produced by a range of carcinogens, including aflatoxin. Because the papers reported only on the work delineating the effect of aflatoxin of p53, they do not conclusively link the incidence of PLC with either suspected etiological agents.

The papers provide supporting evidence for the possibility that p53 mutations caused by aflatoxins or other environmental carcinogens might contribute to the high incidence of PLC in other areas. In the discussion of such an association with aflatoxin, the authors point out that the integration of HBV DNA is a frequent event associated with PLC. A few of these integrations induce insertional mutations in host genes, but are unlikely to cause point mutations, as observed in the reported work with aflatoxin. Before any definitive conclusions can be drawn, further work will be necessary, particularly on the effects of HBV in various hepatoma cell lines.

III. Aflatoxicosis of Animals

The major target organ for aflatoxins in animals is the liver. These compounds have been found to be carcinogenic and teratogenic in animals, as well as the cause of impairment of protein formation, coagulation, weight gain, and immunogenesis.

Domesticated animals are variably susceptible to aflatoxins; some oral LD_{50} values are given for aflatoxin B_1 in several animal species in Table 1. Sheep and cattle appear to be more resistant than most livestock and poultry, whereas swine, chickens, turkeys, and ducklings are more susceptible (Pier 1981). Within a species, there are breed differences; this is best demonstrated in breeds of chickens (Dalvi 1986). Although these factors influence the toxic effects of aflatoxin, other factors to consider are age, sex, exposure (duration and dose), nutrition, environmental stresses, and other chemical exposures including other mycotoxins.

Table 1. Oral LD_{50}'s of Aflatoxin B_1

Animal	Sex	Age/Size	LD_{50} (mg/kg)
Duckling	M	1 d	0.37
Rat	M-F	1 d	1.0
Rat	M	21 d	5.5
Rat	F	21 d	7.4
Rat	F	150 g	17.9
Dog	M-F	Adult	ca. 0.5
Hamster	M	30 d	10.2

A. Swine

Liver changes in swine with acute aflatoxicosis include centrolobular congestion and hemorrhage with the hepatocyte nuclei showing the margination of chromatin and some pyknosis (Cysewski et al. 1968). These changes were followed by centrolobular necrosis and were concomitant with increases in serum enzymes (OCT and AST) and prothrombin time and decreased liver function as measured by bromosulphaphthalein clearance. Pigs so affected became lethargic, did not eat, and eventually became hypothermic, icteric, and had blood in their feces. Additional studies have demonstrated that the major change observed in swine aflatoxicosis is damage to the hepatic parenchyma (Panangala et al. 1986; Miller et al. 1981, 1982; Cook et al. 1989). Panangala and coworkers (1986) found that weanling swine fed 500 ppb dietary aflatoxin had reduced growth rate, feed efficiency, and general unthriftiness. They felt that feeds containing 300 ppb aflatoxins could affect growth rate with prolonged feeding. These studies agree with those of Duthie et al. (1966), Southern and Clawson (1979), and Sisk and Carlton (1972) and influenced the decision of the FDA in establishing 200 ppb as the guideline for aflatoxin in corn for finishing swine. The aflatoxins are not known to significantly affect reproduction in swine (Armbrecht et al. 1972; Hintz et al. 1967). However, as discussed later in this chapter, aflatoxins in sow's milk can affect piglets and *in utero* exposure can affect the integrity of the immune system in neonatal pigs.

B. Cattle

The acute intoxication of cattle with aflatoxins produces liver lesions similar to those described for swine, although cattle are less susceptible to the aflatoxins (Pier et al. 1976; Bodine and Mertens 1983). Affected animals may exhibit reduced feed consumption, weight loss, and reduction in milk production. Masri and coworkers (1969) found that dietary aflatoxin could induce a 33% decrease in milk yields of dairy cattle.

The more significant effects in cattle may occur when animals consume lower levels of aflatoxins and chronic effects appear such as reduced reproductivity, immunosuppression, and reduced feed efficiency. These effects are economically important to the producer, as demonstrated by Guthrie (1979) in which a dairy herd fed 120 ppb aflatoxin-contaminated corn had a 2% reduction in breeding efficiency over a 5-mon period and milk production increased 28% when the cows were given a noncontaminated diet. General health problems occurred in this herd, including small calves, diarrhea, mastitis, reduced feed consumption, and respiratory illness.

Dairy cattle convert dietary aflatoxin rather efficiently to aflatoxin M_1 that occurs in milk. Aflatoxin at 30 ppb in feed will result in milk residue levels of less than 1 ppb (Park and Pohland 1986; Price et al. 1984; Applebaum et al. 1982). Aflatoxin M_1 can occur at 1-hr postdosing in a dairy cow given 0.5 mg aflatoxin B_1/kg of body weight (Trucksess et al. 1983). Mertens (1979) determined that about 0.9% of ingested aflatoxin is converted to aflatoxin M_1 in milk. Similar results were obtained by Van Dijk and colleagues (1984). This metabolic product is slightly less toxic than aflatoxin B_1 (CAST 1989). However, Van Dijk and colleagues (1984) did not find physiologic or biochemical differences in neonatal dairy calves fed milk containing levels of aflatoxin M_1 approximating that which would occur naturally.

Because aflatoxin remained in the rumen content of steers after a 2.5 wk withdrawal period following their being fed approximately 460 ppb dietary aflatoxin for 15 wk, Richard and colleagues (1983) suspected that the rumen function may have decreased. When this hypothesis was subsequently tested using radiotelemetric procedures, they found that rumen motility was reduced in steers given single doses of aflatoxin (Cook et al. 1986). Earlier work had demonstrated that aflatoxins affected *in vitro* and *in vivo* rumen functions such as cellulose digestion, volatile fatty acid formation, and proteolysis (Fehr and Delage 1970; Dvorak et al. 1977).

Thus, the general effect of aflatoxin in cattle is liver disease, with specific effects ranging from acute to chronic disease dependent on the dosage given. Milk production in cows appears to be affected even by low levels of dietary aflatoxins that have no other effects on the health of the animals (Patterson and Anderson 1982). Aflatoxin M_1 can occur readily in the milk of dairy cows given dietary aflatoxin and can cause disease in calves consuming contaminated milk, providing there is sufficient level of contamination (Van Dijk et al. 1984). The FDA has established a 100-ppb guideline level for aflatoxin in corn for breeding cattle, but a 20-ppb limit for corn fed to dairy cattle (CAST 1989).

C. Poultry

The poultry industry is probably more severely affected than any of the livestock industries because poultry appear to be more susceptible to the effects of dietary aflatoxins and losses can be quite severe (Hamilton 1971).

Susceptibility to aflatoxins varies in chickens, depending on dosage, nutritional status, environmental stress, breed, strain, and sex. Low concentrations of dietary aflatoxins can cause weak chickens with reduced weight gains, feed efficiency, and egg production. The latter was noted in at least two studies (Garlich et al. 1973; Wolzak et al. 1985). Again, the primary damage is to the liver, which may be pale, mottled, and have petechial hemorrhages. There may be swelling and vacuolation of hepatocytes and bile duct proliferation. Aflatoxin is a probable cause of fatty liver syndrome in laying hens, a disease causing considerable loss in some large laying flocks (Hamilton and Garlich 1971). Concomitant with pathologic changes in the liver, there are increases in liver-specific serum enzymes.

Although turkeys appear to be more susceptible to aflatoxins than chickens, the effects of aflatoxins on this species are similar to those found in chickens (Richard et al. 1986). Mortalities can occur in poults due to acute hepatic necrosis and hemorrhage after consumption of 1 ppm dietary aflatoxin (Pier 1981).

Of all avian species tested, ducklings appear to be the most susceptible to aflatoxins and, for this reason, they are used in the bioassay for aflatoxins. Acute aflatoxicosis of ducklings is similar to that in turkeys and chickens.

Kratzer et al. (1968) reported that levels of dietary aflatoxin of 1,600 ppb fed for 8 wk did not produce detectable residues in the edible meat of broilers. Similarly, no detectable aflatoxin was found in the meat or eggs of laying hens fed 2,700 ppb aflatoxin for 48 d. In the same study, Rhode Island red chicks were found to be more susceptible than Arbor-Acres hybrid chicks.

D. Carcinogenicity in Animals

The toxicity and carcinogenicity of aflatoxin B_1 are influenced by a variety of factors including, among others, diet, microsomal activity, age, sex, and animal species. The recipient of the toxic insult metabolizes or activates the aflatoxins primarily by the microsomal mixed-function oxidase system of the liver (Shimada and Guengerich 1989). The parent compound is normally detoxified by conversion to hydroxylated intermediates (e.g., aflatoxins Q_1, M_1, and B_2a) that are water-soluble and eliminated via urine or bile. However, certain aflatoxin B_1 intermediates may be formed, such as the 8,9 epoxide (Baertschi et al. 1988), that react with macromolecules (DNA, RNA, and protein) and form adducts (Busby and Wogan 1981). The major DNA adduct formed both *in vivo* and *in vitro* in this reaction is 8,9-dihydro-8-(N7-guanyl)-9-hydroxy aflatoxin B_1 (Gopalakrishnan et al. 1990). These adducts are suspect in the toxicity, especially mutagenicity and carcinogenicity, of aflatoxins in animals (Busby and Wogan 1981). The carcinogenicity of aflatoxins has been demonstrated in a variety of animal species; ducks

given 0.035 ppm aflatoxin developed hepatic tumors in 14 mon (Carnaghan 1965). Trout hepatomas were demonstrated in 96% of the fish fed 20 ppb aflatoxin for 20 mon (Halver 1969). All male rats given 15 ppb dietary aflatoxin B_1 for 68 wk developed hepatocellular carcinomas, whereas the same was true for female rats fed the same diet for 80 wk (Wogan and Newberne 1967). Since these early studies, neoplasms have been induced by aflatoxins in other species such as ferrets, guppies, mice, monkeys, and sheep (CAST 1989). Shalkop and Armbrecht (1974) did report aflatoxin induced hepatic cell adenomas in brood sows fed aflatoxins for 20 mon. However, the importance of carcinogenicity in livestock is diminished because of the relatively short time that these animals are likely to be fed a contaminated diet prior to the time they are marketed for slaughter.

E. Aflatoxin Interactions

The probability that more than one toxin may interact in producing toxicity in animals is high for the following reasons:

1. Several toxigenic fungi often occur in the same batch of feed.
2. Some fungi are capable of simultaneously producing more than one mycotoxin in a substrate.
3. Multiple mycotoxins produced by different fungi have been found in feeds.

Most of the efforts to systematically study some of these interactions have been performed in poultry and certain laboratory animals, as well as swine. Combinations of aflatoxins and several other mycotoxins have been studied. Aflatoxin and T-2 toxin interacted synergistically when given in combination to broiler chicks at dietary concentrations of 2.5 and 4 μg/g, respectively (Huff et al. 1988), as demonstrated by decreases in body weight; increases in the relative weights of the kidney, gizzard, and heart; and decreases in mean corpuscular volume and serum potassium levels.

An important event occurred, with significant application to the diagnosis of aflatoxicosis, when aflatoxin and ochratoxin A were fed to broiler chickens (Huff and Doerr 1981). Although dietary aflatoxin at 2.5 μg/g caused lipid accumulation in the liver, a diagnostic feature of aflatoxicosis in poultry, this phenomenon was inhibited by the simultaneous feeding of 2.0 μg/g of ochratoxin A. This is significant when the diagnosis of aflatoxicosis is made based on signs of the disease (i.e., fatty liver) in poultry. Also, these concentrations of dietary mycotoxins were synergistic in producing increased kidney and gizzard weights relative to body weights. When the same concentrations of these toxins were tested in broilers for the effects on immunity, only complement activity was affected but was likely attributed to the aflatoxin only (Campbell et al. 1983). Similarly, the body weight depression of broilers by this toxin combination persisted longer after a with-

drawal period than did that induced by the individual toxins (Huff et al. 1983). Increased susceptibility to bruising was noted by these workers in birds given these toxins individually and in combination. These authors have studied aflatoxin in combination with deoxynivalenol (Huff et al. 1986) and found no synergistic activity in broilers in several measured parameters.

F. Immunosuppression

One of the major economic considerations of the aflatoxin problem is that of impairment of immunogenesis and native resistance in animals. Within the first decade of research following the recognition of aflatoxins, work had been published on the effect of these toxic metabolites on various immune phenomena (Richard et al. 1978).

One of the initial studies demonstrated that antibody formation in vaccinated mice was affected, whereby typhoid agglutinin titers were depressed (Galikeev et al. 1968). Neither agglutinins nor precepitins to *Pasteurella multocida* in turkeys fed aflatoxin B_1 during or after vaccination were affected (Pier and Heddleston 1970). Similar results were found in the hemagglutinins to *Salmonella pullorum*, as measured by an indirect hemagglutinin test, in chicks given aflatoxin (Adinarayanaiah et al. 1973). Aflatoxin did not affect the hemagglutination inhibition or serum neutralization titers to Newcastle disease virus in turkey poults (Pier et al. 1971). Guinea pig titers to *Brucella abortus* agglutinin titers were not affected by aflatoxin ingestion (Thurston et al. 1974). The depression of agglutinin titers to sheep erythrocytes was found in chicks dosed with aflatoxin (Thaxton et al. 1974). The type of antigen and involvement of cells may be the cause for variation in the antibody effects due to aflatoxin. More recently, studies demonstrated that antibody production was decreased when directed against T-dependent antigen and sheep red blood cells, but was unaffected in animals challenged by T-independent antigens, such as lipopolysaccharide (Reddy et al. 1987).

Certain nonspecific humoral factors associated with a variety of immune phenomena have been affected by aflatoxins and may be related to the ability of aflatoxins to interfere with protein synthesis. Complement activity was depressed in guinea pigs dosed with aflatoxins (Thurston et al. 1972). The activity of this serum component has been depressed by aflatoxin also in poultry, swine, and cattle (Richard 1991). Guinea pigs and cattle given aflatoxins also have a decreased bacteriostatic serum factor to *Escherichia coli* (Thurston et al. 1986). An unknown nonspecific humoral factor appeared to be affected in the aflatoxin-impaired acquired immunity to *P. multocida* in turkey poults because serum from normal or immunized turkeys was beneficial in overcoming the impairment (Pier et al. 1971).

The effects of aflatoxins on the resistance of animals to infectious disease agents are somewhat variable-dependent on organism, toxin dose and constitution, animal species, and perhaps sensitivity of the test. Table 2 lists the various parameters that have been tested in this regard. Doerr and colleagues (1987) found that chickens given aflatoxins were more sensitive to the virulence factors beta hemolysins of the pathogen *Staphylococcus*.

Table 2. Aflatoxin-Induced Changes in Response of Animals to Infectious Disease Agents

Organism	Animal Species	Response	Reference
Candida albicans	Chickens	Increased susceptibility	Hamilton and Harris (1971)
Aspergillus fumigatus	Turkeys	No change	Richard et al. (1973)
Eimeria tenella	Chickens	Increased susceptibility	Edds et al. (1973) Wyatt et al. (1975)
Salmonella gallinarum	Chickens	No change	Smith et al. (1969)
Salmonella spp (4)	Chickens	Increased susceptibility	Boonchuvit et al (1975)
Mycobacterium paratuberculosis	Hamsters	No change	Larsen et al. (1975)
Erysipelothrix rhusiopathiae	Swine	Immunosuppression	Cysewski et al. (1978)
Treponema hyodysenteriae	Swine	Increased susceptibility	Joens et al. (1981)
Streptococcus sp and *Staphylococcus*	Bovine	Increased mammary inflammation	Brown et al. (1981)
Newcastle disease virus	Chicken	Increased susceptibility	Byong and Rothenbacker (1982)
Infectious bronchitis virus	Chicken	Increased susceptibility	Ratanesthankul (1983)

The likely importance of aflatoxins regarding immunity is that they affect the cell-mediated responses. These effects are apparently related to functional aspects of the involved cells, such as lymphokine production and the processing of antigens by macrophages (Pier and McLoughlin 1985). Aflatoxin-induced reduction in the capacity of macrophages to phagocytize particles has been demonstrated (Michael et al. 1973; Richard and Thurston 1975). Richard and Thurston (1975) found that a heat-stable serum substance important in phagocytosis was reduced in rabbits given aflatoxin orally for 3 wk.

The functional aspects of lymphocyte responses to mitogens and production of lymphokines involved in migration inhibition are reduced by aflatoxins (Pier 1986). Similarly, the graft vs host response was reduced in chickens given aflatoxin (Giambrone et al. 1978). Changes in T- and B-cell populations were not found in the peripheral blood of guinea pigs given aflatoxin (McLoughlin et al. 1984). Lymphocytes with HLA-A3 antigen had a lower natural killer activity toward target tumor cells and were more highly suppressed with aflatoxin B_1 than control cells when stimulated by phytohemagglutinin (Cheng-ya et al. 1987).

The proximal entity in a number of the effects of certain mycotoxins on animal systems is unknown, but differences in effects on the immune process among aflatoxin B_1 and its metabolites do exist (Bodine et al. 1983). Aflatoxin Q_1 was active in suppressing *in vitro* lymphoblastogenesis using bovine lymphocytes. Others have found similar results with porcine lymphocytes (Yang 1983).

Immunosuppression has been demonstrated in animals following embryonic exposure. Aflatoxin was transferred across the porcine placenta, and the *in utero* exposed piglets sensitized with killed *Mycobacterium paratuberculosis* had a reduced delayed cutaneous hypersensitivity when skin-tested with johnin 2 wk later (Pier et al. 1985). When chicken embryos were dosed with 0.1 μg of aflatoxin B_1, the graft vs host response was reduced when assayed with a splenomegaly assay (Dietert et al. 1985). There was also a depressed cutaneous basophil hypersensitivity to injected PHA-P.

Considering that there are variations among the results of the studies on aflatoxin-impaired immunogenesis, there are generalized statements that have (Pier and McLoughlin 1985; CAST 1989) and can be made. The aflatoxins impair immunity without suppression of antibody synthesis, suppress nonspecific humoral factors involved in immunogenesis, inhibit phagocytosis by macrophages, cause thymic aplasia, and suppress cell-mediated immunity.

Summary

Aflatoxins remain as a threat to the health of livestock as well as humans by their continuing intermittent occurrence in both feeds and foods. The finding that aflatoxin-contaminated feeds, and eventually purified aflatoxins, were carcinogenic in rats and trout initiated a multitude of studies in search of the role of these toxins in human liver disease, especially cancer. Although aflatoxins have caused acute liver disease in humans, epidemiologic evidence of the involvement of aflatoxins in PLC has not been clarified. Earlier studies did not consider that the hepatitis B virus (HBV) may have contributed to the PLC in the selected populations. Although later studies that did include measurement of the HBV antigen in serum provided conflicting evidence for the role of aflatoxin in PLC in these populations, the latest and most comprehensive study found no association between aflatoxin exposure and PLC mortality. The technological advances and findings of the chemical, immunologic, and metabolic activities of aflatoxins such as binding to DNA and protein to form adducts, development of monoclonal antibodies, and mutational specificity of the genotoxic compounds will, it is hoped, help to clarify the role of aflatoxin as a risk factor, among many others, in the development of primary liver cancer in humans.

Aflatoxicosis of animals is ususally manifested by pathologic changes in the liver, but they have been found to be carcinogenic and teratogenic as well as causing impaired protein formation, coagulation, weight gains, and immunity. The importance of the carcinogenic effect in livestock is diminished because they are not fed contaminated diets for a sufficient time prior to marketing for slaughter. Animals are variably susceptible to aflatoxins, depending on such factors as age, species, breed, sex, nutrition, and certain stresses.

Swine, cattle, and poultry are the domestic species of greatest economic concern in terms of aflatoxicosis. In all species, the evidence of disease is a general unthriftiness and reduction in weight gains, feed efficiency, immunity, and production. More conclusive evidence of aflatoxin involvement in disease includes acute to chronic liver disease with concomitant increases in specific liver enzymes in the serum. In cattle, milk production is affected, but of greater significance is that the aflatoxins in feeds can be rather efficiently converted to toxic metabolites in milk, with even small amounts being readily detectable. The poultry industry probably suffers greater economic loss than any of the livestock industries because of the greater susceptibility of their species to aflatoxins than other species.

Because other mycotoxins may occur in feeds in concert with aflatoxins, a consideration of their possible interactions in producing disease is important. The aflatoxins have been shown to interact synergistically with both T-2 toxin and ochratoxin in their effects on poultry.

Immunosuppression is a major economic effect of aflatoxins. Although antibody production is impaired when high concentrations of aflatoxins are consumed, the major effects are related to cellular immune phenomena or the reduction of nonspecific humoral factors such as complement and interferon. They cause thymic aplasia and inhibit phogocytosis by macrophages, delayed cutaneous hypersensitivity, graft vs host response,and interfere with lymphoblastogenesis and leukocyte migration. The effects of aflatoxins on infectious diseases are dependent on the disease agent, toxin dose and constitution, animal species, and perhaps sensitivity of the test. The embryonic exposure of animals to aflatoxin occurs and can affect the immune status of a neonatal animal.

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